Journal of Molecular Catalysis B: Enzymatic 61 (2009) 268–273

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

α- and β-oxygenated aldehydes derived from Diels–Alder reactions as substrates for hydroxynitrile lyases

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ARTICLE INFO

Article history:
Received 2 June 2009
Received in revised form 10 August 2009
Accepted 12 August 2009
Available online 19 August 2009

Keywords:
Cyanohydrins
Enzyme catalysis
Hydroxynitrile lyases
Molecular modelling
Biocatalysis

ABSTRACT

3,4-Dihydro-2H-pyran-2-carbaldehyde (**1**) and 2-methoxycyclohex-3-encarbaldehyde (**2**) obtained by thermal or chemocatalytic Diels–Alder reactions were converted into the corresponding cyanohydrins by hydroxynitrile lyase catalysis. Modelling investigations give a clear interpretation for the steric course of the biocatalytic cyanohydrin reaction of these α- and β-oxygenated aldehydes.

1. Introduction

Biocatalysts are of increasing importance for the pharmaceutical and agrochemical industry for the synthesis of enantiopure complex molecules [1]. Especially C–C bond forming enzymes are gaining momentum in the last years [2,3]. Hydroxynitrile lyases (HNLs), which catalyse the enantioselective synthesis of cyanohydrins, for example find application in the production of valuable precursors for many pharmaceuticals and agrochemicals, as ACE-inhibitors and pyrethroids [3,4].

Recently, α- and β-oxygenated aldehydes were investigated as substrates for the HNLs from *Prunus amygdalus* (*Pa*HNL) and *Hevea brasiliensis* (*Hb*HNL) [5–7]. α-Oxygenation influences strongly the HNL catalysed transformation. The selectivity decreases in comparison to the corresponding alkylated aldehydes. Most of these substrates also had an additional stereocentre adjacent to the carbonyl group, but the HNLs employed showed no chiral discrimination [5–7].

Aldehydes **1** and **2** (see Fig. 1) were available by Diels–Alder reactions from a cooperation project within the CERC-3 framework. The joint structural element is an oxygen functionality α and β to the carbonyl group. This opened the opportunity to extend the substrate spectrum of the biocatalytic cyanohydrin reaction and to finalize earlier work [5–7] on the influence of α- or β-oxygenation on this transformation both by experimental work and by molecular modelling.

2. Experimental

All solvents and materials not described in this chapter are commercially available and were appropriately purified, if necessary. The hydroxynitrile lyases were kindly provided by DSM Fine Chemicals Austria. Reactions were monitored by TLC (Merck silica gel 60 F_{254} or aluminium oxide 60 F_{254} neutral) and the compounds were visualised by spraying with Mo-reagent (10% H_2SO_4, 10% $(NH_4)Mo_7O_{24}{\cdot}4H_2O$ and 0.8% $Ce(SO_4)_2{\cdot}4H_2O$ in water) or vanillin/H_2SO_4 solution (1 g vanillin in 1000 mL H_2SO_4 conc.). Flash chromatography was performed on Silica gel 60 (70–230 mesh, Merck). ^1H and ^{13}C NMR spectra were recorded on a VARIAN INOVA 500 MHz or a Bruker AMX 500 MHz spectrometer (^1H 500 MHz, ^{13}C 125 MHz) with TMS as an internal reference. NOE spectra were recorded on a Bruker AC 250 MHz spectrometer. Enantiomeric purities were analysed using a Hewlett Packard 6890 instrument equipped with a FID and a Chirasil-DEX CB column (25 m × 0.32 mm, 0.25 μm film). GC/MS measurements were per-

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doi:10.1016/j.molcatb.2009.08.005

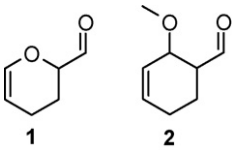

Fig. 1. Substrates.

formed employing a Hewlett Packard HP6890 Series-II GC system equipped with a HP 5973N mass selective detector with methane as reactant, a HP5-MS column (30 m × 0.25 mm, 0.25 μm film) and He as carrier gas. For analytical data *vide infra*.

2.1. Synthesis and safe-handling of anhydrous HCN—caution

All reaction equipment, in which HCN or cyanides were involved, was placed in a well ventilated hood. For continuous warning, an electrochemical sensor for HCN detection was used. The required amount of HCN was freshly prepared by adding dropwise a saturated NaCN solution to aqueous sulphuric acid (60%) at 80 °C. HCN was transferred in a nitrogen stream through a drying column and collected in a cooling trap at −12 °C. Waste solutions containing cyanides were treated with aqueous sodium hypochlorite (10%). Subsequently the pH was adjusted to 7.0 with aqueous sulphuric acid.

2.2. 3,4-Dihydro-2H-pyran-2-carbaldehyde (**1**)

The synthesis was performed according to literature and the spectroscopic data are identical with those reported [8]. The ratio aldehyde **1**/acrolein after purification is 5/1.

2.3. Procedure for the synthesis of 2-methoxycyclohex-3-enecarbaldehyde (**2**) by thermal Diels–Alder reaction

To a solution of acrolein (1.5 equiv.) in toluene a small amount of *p*-hydroquinone and 1-methoxy-1,3-butadiene (1 equiv.) is added. The mixture is heated to reflux until quantitative conversion. After cooling to rt the solution is filtered and the solvent and excessive acrolein are evaporated under reduced pressure yielding the crude Diels–Alder adduct **2** (81%, *cis*/*trans* = 6/1) as a light yellow liquid. Separation of the *cis*- and *trans*-product is achieved by HPLC (eluent: hexane/ethyl acetate = 85/15). Spectroscopic data are slightly different from literature [9]. ^{1}H NMR ($CDCl_3$) *cis*: δ 9.75 (d, J = 1.10 Hz, 1H, CHO), 5.96–5.88 (m, 2H, H3, H4), 4.05 (m, 1H, H2), 3.32 (s, 3H, OMe), 2.49–2.44 (m, 1H, H1), 2.20–2.10 (m, 1H, H5), 2.00–1.90 (m, 1H, H5′), 1.90–1.75 (m, 2H, H6); *trans*: δ 9.74 (d, J = 1.51 Hz, 1H, CHO), 5.84–5.76 (m, 2H, H3, H4), 4.05 (m, 1H, H2), 3.34 (s, 3H, OMe), 2.57–2.51 (m, 1H, H1), 2.07–2.01 (m, 1H, H5), 2.00–1.75 (m, 3H, H5′, H6). ^{13}C ($CDCl_3$) *cis*: δ 203.7 (CO), 132.2, 125.0 (olefinic), 72.6 (C2), 56.4 (OCH_3), 50.2 (C1), 23.9 (C6), 18.2 (C5); *trans*: δ 203.6 (CO), 132.6, 124.4 (olefinic), 73.2 (C2), 56.9 (OCH_3), 51.3 (C1), 24.4 (C6), 17.7 (C5). MS: 141 (M^+), 109 (M-MeO), 81 (M^+-(MeO + CO)), 79 (C_6H_7).

2.4. Procedure for the synthesis of 2-methoxycyclohex-3-enecarbaldehyde (**2**) catalysed by salen–chromium complexes

The reaction was performed under anhydrous conditions. To a solution of Jacobsen's salen–chromium complex [10] (63 mg, 5 mol%) or polyglycerol-supported salen–chromium complex [11] (1.41 mmol g^{-1}, 78 mg, 2.2 mol%) in anhydrous CH_2Cl_2 (1 mL/mmol) 4 Å molecular sieves is added and the mixture is cooled to 0 °C. Subsequently 1-methoxy-1,3-butadiene (1 equiv.) and acrolein (1 equiv.) are added and the mixture is stirred for 24 h (Jacobsen's salen) or 48 h (polyglycerol-supported salen) at 0 °C. The solvent was removed in vacuo and the residue was purified by column chromatography (hexane/ethyl acetate = 9/1) to yield the *cis*/*trans*-mixture of **2** in a ratio of 9/1.

2.5. General procedure for the synthesis of racemic cyanohydrins (blank reaction)

To a solution of aldehyde in *tert*-butyl methyl ether (*t*BME) an aqueous buffer (30 mM, citrate, 1/1, v/v) is added. The resulting mixture is stirred at 0 °C until an emulsion is formed. After addition of freshly prepared prussic acid (3.6 equiv.), the mixture is stirred at 0 °C until quantitative conversion. The emulsion is broken with Celite 545, filtered and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yields the crude cyanohydrins as light yellow liquids. For determination of the product distribution a small amount is acetylated with acetic anhydride and pyridine in dichloromethane using standard methods.

2.6. General procedure for the enzymatic synthesis of cyanohydrins

Method A: HbHNL. To a solution of aldehyde in *t*BME an aqueous solution (1/1, v/v), containing particular amounts of *Hb*HNL (approx. 4000–5000 U/mmol aldehyde) in buffer (30 mM, citrate, pH 5.0), is added and the resulting mixture is stirred at 0 °C until an emulsion is formed. After addition of freshly prepared prussic acid (1.8–3.6 equiv.), the mixture is stirred at 0 °C until quantitative conversion. The emulsion is broken with Celite 545, filtered and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yields the crude cyanohydrins as a light yellow liquid. For the determination of the distribution of the stereoisomers, a small amount is acetylated.

Method B: PaHNL. To a solution of aldehyde in *t*BME an aqueous solution (1/1, v/v), containing particular amounts of *Pa*HNL (approx. 2000–2500 U/mmol aldehyde) in buffer (30 mM, citrate, pH 5.0), is added and the resulting mixture is stirred at 0 °C until an emulsion is formed. Freshly prepared prussic acid (2.0–3.6 equiv.) is added and the mixture is stirred at 0 °C until quantitative conversion. The emulsion is broken with Celite 545, filtered and dried over Na_2SO_4. Solvent removal in vacuo gives the crude cyanohydrins as light yellow liquid. For the determination of the distribution of the stereoisomers, a small amount is acetylated using standard procedures.

Table 1
HNL catalysed conversion of **1** to give cyanohydrins **3**.

Entry	Method	pH	Enzyme amount (U/mmol)	HCN (equiv.)	(2*S*,2′*R*)- [a] **3** (%)	(2*R*,2′*R*)- **3** (%)	*de*- **3** (%)	(2*R*,2′*S*)- **3** (%)	(2*S*,2′*S*)- **3** (%)	*de* **3** (%)	Yield (%)
1	*Hb*HNL	4.8	4000	3.6	5.7	43.7	76.9	7.1	43.5	71.9	73
2	*Pa*HNL	3.7	2500	3.6	46.7	4.2	83.5	46.3	2.8	88.6	68
3	Blank	3.2	–	3.6	22.7	27.1	8.8	28.3	21.9	12.7	86

[a] Configuration at position 2′might be exchanged.